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Mixing Operations for 50 L to 2000 L Single-Use Mixer: Liquid-Liquid Mixing Characterization and Slurry Suspension

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ABSTRACT

Mixers are employed throughout all parts of bioprocessing. Single-use mixers (S.U.M.) are commonly used for both upstream and downstream operations. Upstream operations can include media formulation and hydration, media holding, and sterile filtration. Downstream operations can include product storage, viral inactivation, buffer preparation, and slurry preparation for column packing. Correct mixing parameters and control are key elements for successful mixing operations particularly in the transition from non single-use platform to single-use platforms. This study presents the automation of simple mixing procedures using the Thermo Scientific™ HyPerforma™ S.U.M. with Touchscreen Console. Also reported is the selection method for scalable mixing parameters allowing normalized mixing performance across all single-use mixer sizes.

The following applications are demonstrated in this study: Characterization of scalable mixing parameters by comparing power input per volume and T95 blend time criteria, slurry suspension in preparation for chromatography column packing using the 100 L S.U.M., minimum speed for complete slurry suspension is identified as well as quantification of resin damage post mix. This work demonstrates best practices for mixing in bioprocessing unit operations including use of the Touchscreen Console.

INTRODUCTION

Mixing is a unit operation heavily used throughout all steps in bioprocessing. Eliminating a concentration gradient is the primary function of the HyPerforma S.U.M.. Optimal operating parameters are critical for success in scaling and are best practice for mixing. Often when mixers are considered RPM is the only parameter considered when operating and scaling mixing to various sizes.

Gradients can be measured using various sensing methods including RTDs, pH and conductivity meters, metabolite analyzers, etc. Gradients are dissipated by inputting power from a motor into the liquid via an impeller. The amount of power inputted to the liquid by a motor is characterized by the Power Input per Volume (PIV) equation:

$$PIV = \frac{N_p \rho N_i^3 D_i^5}{V}$$

Where N_p is impeller type or power number, ρ is fluid density, N_i is speed of the impeller, D_i is impeller size, and V is the volume of the mixer. The power number N_p is a constant unique to the impeller and can be considered similar to a drag coefficient. We assume a power number for all impellers investigated to be 2.1. The PIV equation is an extremely valuable tool particularly when scaling vessel size and volume. Scaling by PIV provides normalized mixing performance regardless reactor characteristics.

Thermo Scientific HyPerforma S.U.M. with Touchscreen Console offers the following functionality within the bioproduction workflow including:

- Automated BioProcess Container air fill
- Agitation control
- Sterile media filtration with sterile filter monitoring
- Automated liquid fill
- Automated harvest
- pH monitoring
  - acid/base titration control using bolus addition
- Conductivity monitoring
  - Saline titration control using bolus addition
- Pump Control, up to 4 pumps
  - with bolus addition feature interlocked by pH or conductivity process values
- Pneumatic pinch valves for fill and harvest lines
- Temperature monitoring and control
- Mass or volume monitoring
- Data historian to monitor all modules and export through USB or Profibus connection
- User adjustable alarms and interlocks
- Additional analog auxiliary ports for additional transmitters

Applications of the HyPerforma Mixer with Touchscreen panel include:

- Media/buffer hydration
- Sterile media hold tank
- Automated pH and conductivity shifts
- Automatic fill and harvest
- Viral Inactivation
- Slurry suspensions
- Product pooling
- Waste containment
- Bulk storage and final fill
- Robust data collection via historian

Resin preparation for column packing is an audacious time consuming task typically performed in a stainless vessel. Often just cleaning stainless vessels consumes the majority of the operators day. Substituting the stainless mixer with Thermo Fishers HyPerforma S.U.M. allows end users to efficiently mix and store resin at the desired concentration while eliminating costly clean up time. This presentation demonstrates first scalability of the S.U.M. and second the ability of the HyPerfoma S.U.M. to sufficiently mix resin at multiple concentrations and volumes in a 100L S.U.M..

MATERIALS AND METHODS

S.U.M. Mixing Characterization

To test mixer capabilities liquid-liquid mixing was performed on the 50 L, 100 L, 500 L, 1000 L, and 2000 L S.U.M.s. each at full volume, half volume and 5:1 volume. Gradients were formed by manually adding a bolus of concentrated NaCl and measured using conductivity sensors placed at the top, middle and bottom of each vessel. PIV of each vessel was evaluated at max speed, 100 W/m³ and 20 W/m³. Maximum operating speeds for all vessel sizes is 350 RPM.

Table 1. S.U.M. Impeller size

Vessel Size	50 L S.U.M.	100 L S.U.M.	500 L S.U.M.	1000 L S.U.M.	2000 L S.U.M
Impeller Size (cm)	14.6	14.6	20	20	25

S.U.M. Slurry Mixing

Testing was performed on the 100 L S.U.M. to identify minimum power required to keep slurry in suspension at 30%, 50% and 70% concentration. Evaluation of slurry mixing at 20 L, 50 L and 100 L volumes at each respective concentration was also considered. Evaluation consisted of operating the mixer at 10, 20, 30 40, and 50 W/m³ power input at each volume and concentration.

Mixing evaluation consisted sampling at the top, middle, and bottom of the vessel and measuring for conformity. After the mixing regime had been established 50 mL samples were taken and centrifuged for 10 minutes at 1000g. Upon centrifugation, samples were observed for turbidity of fine particulate in the supernatant and the solid concentration was determined using a custom 3D printed slurry concentration measurement tool.

Potential for resin degradation was a concern. Upon identification of the minimum PIV required to keep slurry in suspension the mixer was operated at 150% of minimum PIV and damage to the slurry was measured by microscopy and particles were quantified using a Beckman Coulter Multisizer. Also evaluated was resin damage after long mix times when operated at correct suspension speeds.

RESULTS

S.U.M. Mixing Characterization Results

Conductivity based mixing studies were performed using the 50 L, 100 L, 500 L, 1000 L, and 2000 L S.U.M..

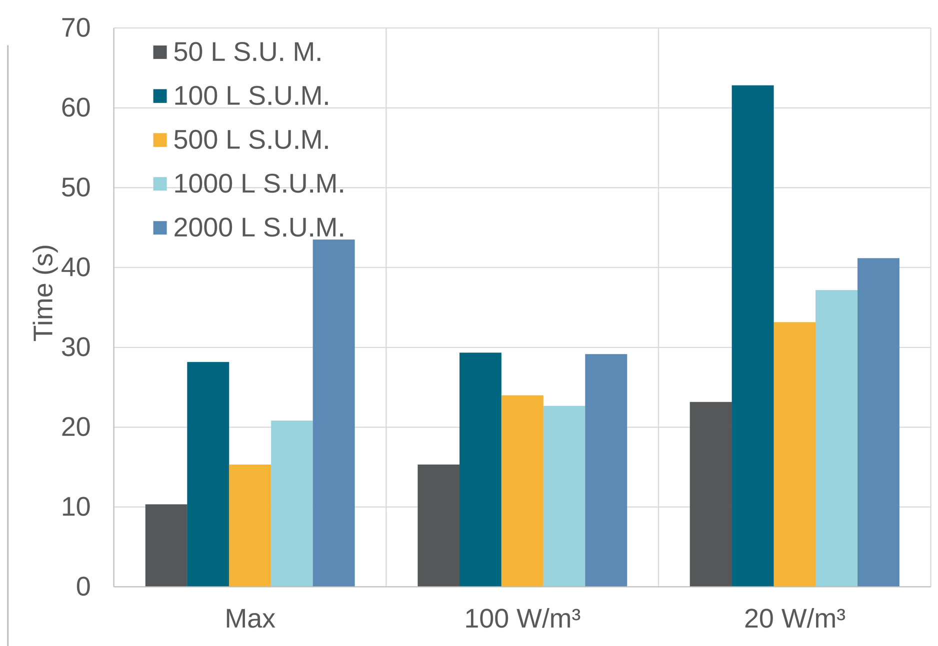


Figure 1. T95 mixing times at full volume in the HyPerforma S.U.M. at maximum PIV, 100 W/m³ and 20 W/m³

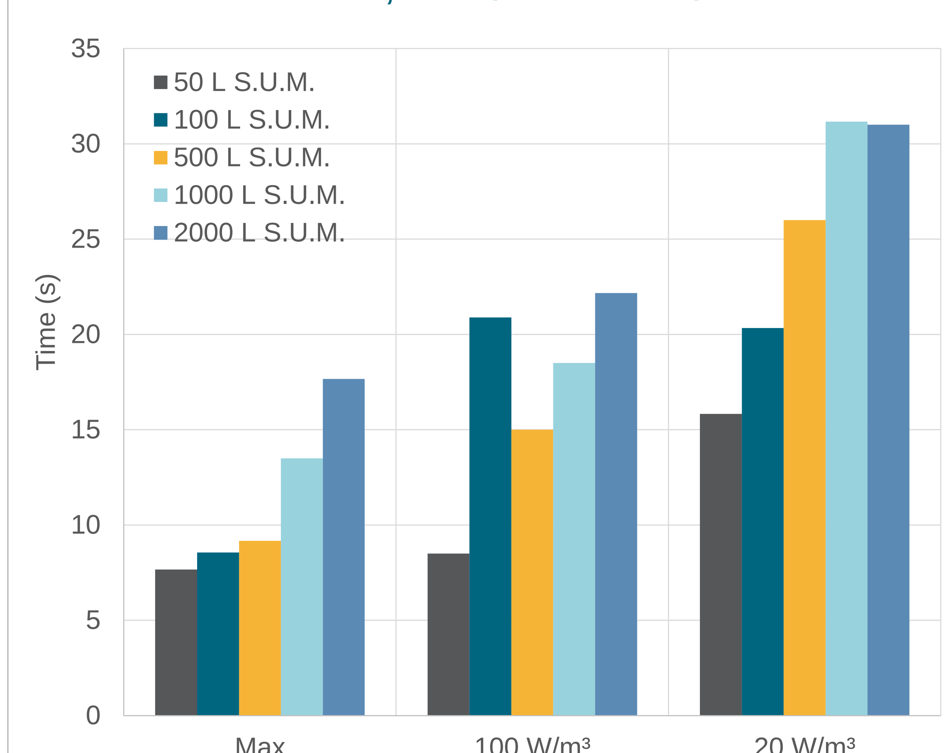


Figure 2. T95 mixing times at 2:1 volume in the HyPerforma S.U.M. at maximum PIV, 100 W/m³ and 20 W/m³

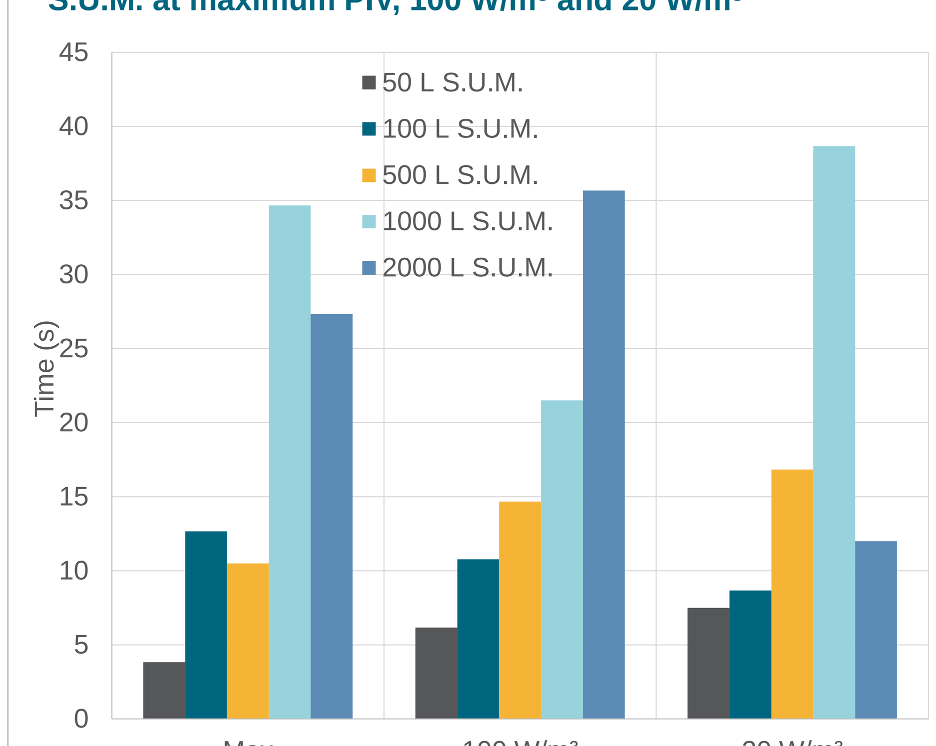


Figure 3. T95 mixing times at 5:1 volume in the HyPerforma S.U.M. at maximum PIV, 100 W/m³ and 20 W/m³

S.U.M. Slurry Mixing Results

Minimum suspension speeds were empirically identified at 30%, 50% and 70% slurry concentrations to occur at a power input of 20 W/m³. No measureable gradient occurred within the 20 W/m³ to 50 W/m³ range of power input.

Figure 4. 100 L S.U.M. 70% slurry concentration

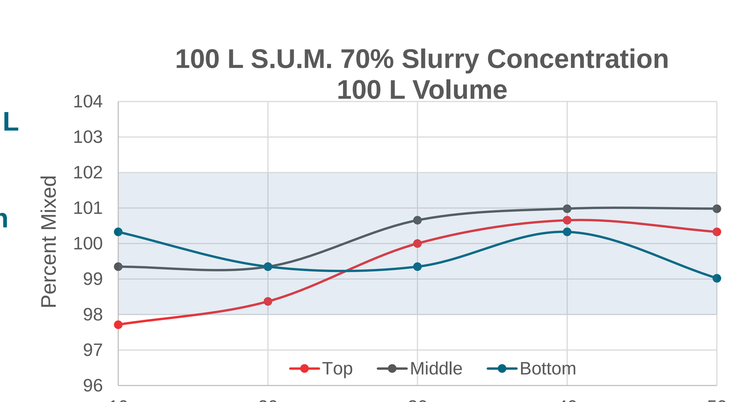


Table 2. Normalized S.U.M operating parameters, RPM requirements for power input vs. volume

HyPerforma S.U.M. 50-2000 L RPM Requirements for Normalized PIV															
	50 L S.U.M.			100 L S.U.M.			500 L S.U.M.			1000 L S.U.M.			2000 L S.U.M.		
PIV (W/M^3)	10 L	25 L	50 L	20 L	50 L	100 L	100 L	250 L	500 L	200 L	500 L	1000 L	400 L	1000 L	2000 L
10	54	73	92	68	92	116	68	93	117	86	117	148	75	102	128
20	68	92	116	85	116	146	86	117	148	109	148	186	94	128	161
30	77	105	132	98	132	167	99	134	169	124	169	213	108	147	185
40	85	116	146	107	146	184	109	148	186	137	186	234	119	161	203
50	92	125	157	116	157	198	117	159	200	148	200	252	128	174	219
60	98	132	167	123	167	210	124	169	213	157	213	268	136	185	233
70	103	139	176	129	176	221	131	178	224	165	224	282	143	194	245
80	107	146	184	135	184	231	137	186	234	173	234	295	150	203	256
90	112	152	191	141	191	241	142	193	244	179	244	307	156	211	266
100	116	157	198	146	198	249	148	200	252	186	252	318	161	219	276

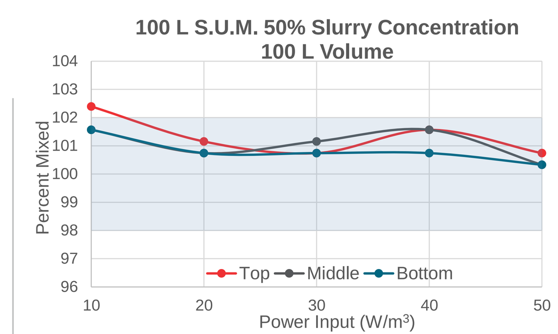
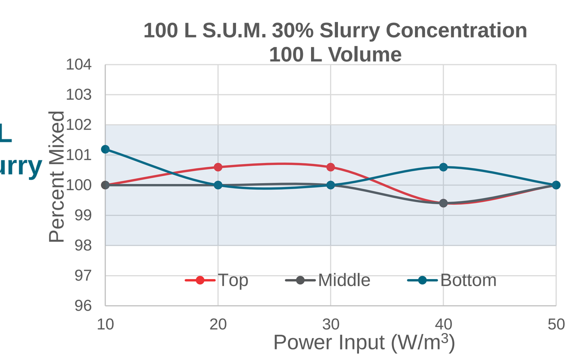


Figure 5. 100 L S.U.M. 50% slurry concentration

Figure 6. 100 L S.U.M. 30% slurry concentration



Post mix resin samples were compared to two conditions, first, a pre mix condition to allow for a baseline comparison; and second, resin that had been intentionally physically damaged to demonstrate behavior of damaged resin. Quantifying damage done to the slurry was difficult to perform with microscopy therefore particle analysis was required.

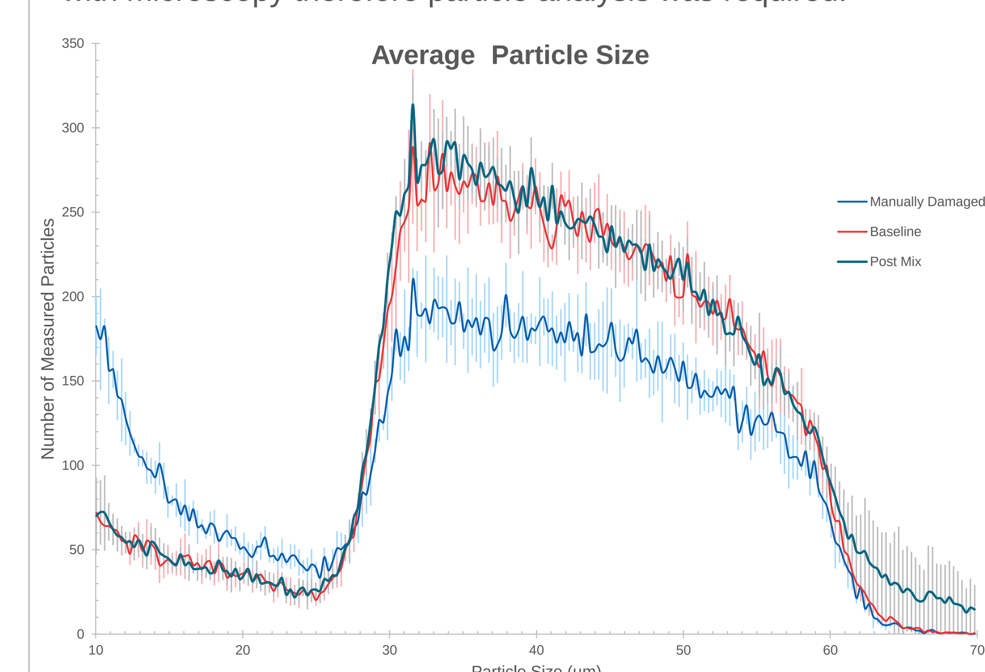


Figure 7. Particle analysis results for suspended slurry

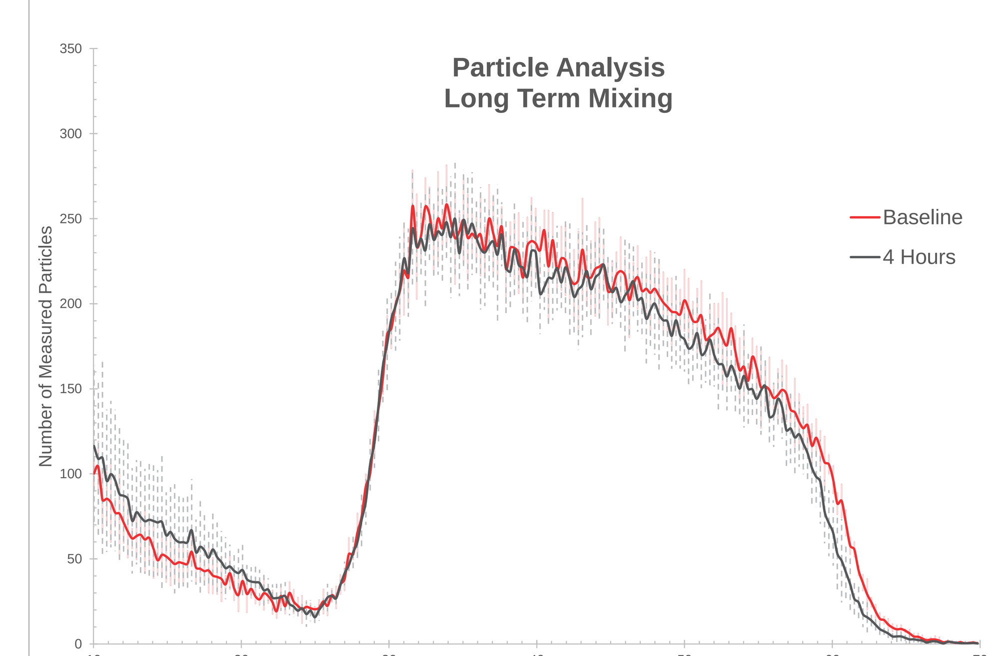


Figure 8. Particle analysis post four hours mixing

CONCLUSIONS

HyPerforma mixers are designed to eliminate gradients in vessels. Correctly operating mixers can maximize mixing performance increasing overall operator efficiency in scaling. Results demonstrate effectiveness of the S.U.M. in scaling based on power input across a wide range of volumes and mixer sizes.

The HyPerforma S.U.M. is capable of mixing slurry at various concentrations and volumes using standard equipment and operating procedures. Operation in the 20 to 30 W/m³ range provides sufficient mixing without damaging resin.

REFERENCES

1. Doran, P. M. Mixing. in *Bioprocess Engineering Principles* 282-285 (Elsevier, 2013).

TRADEMARKS/LICENSING

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